FACTS AND FALLACIES IN DISINFECTION.

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ONE of the most important problems confronting the well informed health officer is that of cleaning up or preventing infection. Whatever measures he takes, these should be supplemented by an intelligent use of some chemical disinfectant. What shall he use? A new substance is proposed and gains a certain degree of popularity only to be relegated to a secondary place in favor of one still newer. One is likely to lose sight of the virtues of the older disinfectants in this maze of new products.

It is advisable to glance over the field occasionally to discover the advantages and disadvantages of these various substances; for while few are valueless only the best for the specific purpose is to be recommended.

A disinfectant is often overrated because in the hands of a careful user exceptionally good results were obtained. Some are discredited because of being used under improper or inappropriate conditions. Almost any substance may, under certain conditions, appear to be a disinfectant when in reality the conditions and not the substance were unfavorable to the development of the organisms.

If bacteria are transplanted from contact with a substance which reduced their vitality, to a culture medium slightly unfavorable for growth, an erroneous impression will often be obtained as to the germicidal value of the substance in question.

This actually occurred in testing a well known protein compound of silver which in a medium regularly adjusted by addition of hydrochloric acid to an acidity of 1.5, gave a phenol coefficient of 5 while in an unadjusted medium with acidity of 0.8 the coefficient fell to about .5 to 1. (See Protocol 1, Appendix.)

Another occasion for the discredit of a good disinfectant is the erroneous idea in the lay mind that a disinfectant is an insecticide. Formaldehyde fails as a fumigant for destroying insect life, a fact which may partly explain the appearance of adverse reports. (See Protocol 2, Appendix.)

The more probable reason, however, for the often expressed disparagement of formaldehyde is an improper use largely because of a misconception of its properties. Dry formaldehyde gas while exceedingly irritating to mucous membrane is almost innocuous to bacteria, the germicidal value being brought out only when in aqueous solution, or probably by the hydrate. It readily polymerizes, so that if slowly generated with insufficient moisture present, the active hydrate is not produced in a proportion to be efficient. (See Protocol 3, Appendix.)

Various improper methods have

been proposed and applied for generating the gas from its aqueous solution. Potassium permanganate or chromate by combining with a portion of the agent generates sufficient heat to volatilize the remainder with the necessary water, the amount destroyed not exceeding 20 to 30 per cent. of the original content, while the rapidity and completeness of evolution both of formaldehyde gas and water vapor more than offset the necessary loss.

When lime is used as the heating agent only the heat of combination with water can be permitted, since the lime-water slowly but almost completely destroys the aldehyde forming inert decomposition compounds. The same is true of caustic soda which is the reagent employed in one or more commercially exploited generators. It is also essentially true if calcium hypochlorite is so employed.

A prompt and more or less violent reaction occurs when a 40 per cent. aqueous solution of formaldehyde is mixed with any one of the above named reagents, but careful experiments failed to find effective quantities of formaldehyde among the evolved gases.

The method employed to determine the amount evolved was essentially that described by Frankforter (J. A. C. S., Vol. 28, 1906, p. 1234) the gases being absorbed by an excess of distilled water. By this method 30 per cent. of the theoretical quantity of gas was found to be liberated from a solution by means of potassium permanganate, while only 3 per cent. could be found when caustic soda was the reagent used. Lime is more efficient than caustic soda because a higher degree of heat is evolved in the reaction. Chlorinated lime under the same conditions appeared to evolve only chlorine compounds, no formaldehyde gas being detected in the aqueous solution collected.

Any rapid method by which the unchanged formaldehyde gas can be driven off from its aqueous solution is more or less satisfactory as a means of disinfecting a room by fumigation.

Formaldehyde gas, however, is so volatile that it must be almost immediately redissolved in water vapor or it will be dissipated before an effective strength is obtained. Therefore there are three highly essential factors in a successful fumigation with formaldehyde, namely, very rapid evolution of gas, a percentage of moisture in the air approaching saturation and absence of air currents. It follows that disinfection should not be attempted on a windy day, that the air must be rendered moist by sprinkling the floor with water about fifteen minutes before fumigation and if possible that a permanganate or chromate salt be used to develop the requisite heat.

Calcium hypochlorite or bleaching powder is one of the very few substances which spontaneously evolves an efficient disinfecting vapor.

The gas first released is chlorine which has a very caustic action on fabrics, colors and metals, and is therefore objectionable in most cases.

Hydrogen peroxide has had a varied reputation. Reputed very highly by some, it is considered worthless by

others. It all depends on the point of view and the way in which it is used.

With a phenol coefficient of .03 it is as strong as a 3 per cent. solution of phenol and therefore theoretically capable of destroying almost all infectious organisms. Practically, however, it is so rapidly destroyed in contact with organic matter that no great dependence can be placed on it as a disinfectant unless used repeatedly in considerable quantities. It has a value, however, all out of proportion to its germicidal action, in that as an active oxylizing reagent, the evolved gas acts as a cleansing agent, the foreign matter in wounds being extruded in a very efficient manner. It is therefore active in proportion to its hydrogen dioxide content but not primarily as to its phenol coefficient.

Much difference of opinion exists regarding the value of soap as a disinfectant. Tested against bacteria by any of the quasi-official methods a pure, neutral soap has little germicidal action in any practicable strength of solution. A 5 per cent. solution of most soaps is efficient in killing all non-spore-bearing bacteria in a few minutes but this is not a practicable solution to obtain except with soft soap.

Many soaps, however, are not neutral but decidedly alkaline and to this ingredient undoubtedly some of the relatively high values may be attributed. (See Protocol 4, Appendix.) A neutral soap or preferably one that is slightly alkaline is indirectly valuable because of its cleansing properties, particularly in its action to remove the protecting fatty film on the skin and allow penetration of an active agent. Theoretically a true disinfectant associated with soap is an ideal combination for cleansing and disinfecting the hands and the site of an operation. Practically, however, few of the disinfectants with which we are familiar can be advantageously used with soap either because of the physical properties of the resulting mixture or of chemical reactions impairing the quality of one or both of the two agents. Symes investigating this subject (Antiseptic and Disinfectant Properties of Soap, 1909) found that a 1 per cent. solution of soap—as strong as an average solution can be made—kills some bacteria in ten minutes, but most are not killed after hours of exposure, which is also true even of a 5 per cent. solution. He also found that no added disinfectant greatly enhanced the value of soap except biniodide of mercury which was very effective for disinfecting instruments and the field of an operation. Plain soap is germicidal toward some bacteria, notably B. *typhosus*. My own experiments (See Protocol 5, Appendix) have demonstrated that the different glycerides produce soaps which differ in their germicidal values, but that no soap can be considered an effective disinfectant unless associated with some other more active agent. The substitution of scrubbing with soap for fumigation in terminal disinfection is not a safe practice. The cleaning is to be commended but is not a substitute; it is only an aid to disinfection with a fumigant.

Ethyl alcohol is very largely used as a disinfectant for the site of an opera-

tion or injection, but by many it is regarded as of little value. It is generally considered that only the medium strengths of alcohol are active.

Experiments recently carried out verify the results obtained by Harrington and Walker (*Boston Medical and Surgical Journal*, Vol. 148, p. 548) (See Protocol 6, Appendix), who found that "unless the bacterial envelope contains a certain amount of moisture it is impervious to strong alcohol; but dried bacteria in contact with dilute alcohol containing from 30 to 60 per cent. of water will absorb the necessary amount of water therefrom very quickly, and then the alcohol itself can reach the cell protoplasm and destroy it."

It is evident, therefore, that sponging the site of an operation with alcohol after scrubbing with soap is a safe procedure; adding acetone to the alcohol is logical as a fat solvent and is quite commonly practiced. A further aid to disinfection is the addition of 1 to 2 per cent. of a high coefficient coal tar disinfectant to the acetone-alcohol mixture by which one can obtain a sterilizing solution equivalent to 40 per cent. phenol. (*Journal of Surgery, Gynecology and Obstetrics*, Vol. 21, p. 85.) Such a solution is neither poisonous nor irritating except when the hands are encased in rubber gloves after being treated with the mixture.

The high coefficient disinfectants of coal tar origin have several factors to contend with in finding a satisfactory position among the known germicidal agents. There is doubt as to the high values assigned to them on the basis of a laboratory test, especially since many coal tar products have been proved to be almost worthless. While it is true that no ideal method of standardization of disinfectants has been evolved many of the variable factors have been discovered and eliminated, so that there is reason to hope that germicidal agents may some day be as accurately graded as other chemical substances. The tests applied are relative, however, and there is little reason to suppose that a substance found by a careful laboratory test to be 2, 5, or 20 times as efficient as another similar substance would not be found in practice to have the same relative value when used under similar conditions. If the standard is of the same character and the conditions are the same the statement could not be questioned. Unfortunately the standard used for comparison is, although technically similar, actually decidedly different in many respects. Pure phenol has been chosen as the standard with which to measure the activity of all classes of disinfectants, and, while it belongs to the same series as the other phenols of coal tar, there are many conditions which change the resistance of the test organism toward everything but the standard. This is a generally recognized fact and is a serious defect.

On the other hand, the conditions imposed on the laboratory test are sufficiently severe as to eliminate most of these variants.

Another defect in the method of grading disinfectants by their phenol coefficients is the fact that different organisms have a ratio of resistance different from that of B. *typhosus*—the

standard test organism. With some organisms the values of disinfectants is higher and with others lower than their present rating. (See Protocol 7, Appendix.) Because of this and other factors involved, the Bureau of Animal Industry has felt compelled to reject all but pharmacopœial germicides for disinfecting premises possibly infected by hog cholera, foot and mouth, and other infectious diseases. This has made necessary the use of the relatively expensive cresylic acid, practically all of which is imported, while thousands of gallons of disinfectants from coal tar distillates of local production are barred from official use. This is a hardship because of the relatively high cost of the imported cresylic acid and is economically unwise because of its relatively low germicidal value. When properly carried out the germicidal test which establishes the phenol coefficient is of more value than a chemical test which compares phenol content only and takes no cognizance of the difference in character of the different classes of phenols. (See Protocol 8, Appendix.)

In connection with the policy of the Department of Agriculture in recommending disinfectants, a point is worthy of notice that lime is recommended to be mixed with the solution of phenol or of cresol compound in the proportion of not to exceed 1½ pounds to 1 gallon of solution. There is unfortunately no express instruction to use fresh water-slaked lime which would have a degree of germicidal value in itself. The lime compounds of phenol and cresol have about one half the germicidal values of the uncombined substances. While the lime mixed with cresol compound not only lowers the value of the cresylic acid, but is itself partly neutralized by the soap, forming a sticky inert lime soap. Such a practice, while intended for the good purpose of showing where the disinfectant has been applied, would appear to do more harm than good unless as noted above the lime used is fresh stone lime. In that case the freshly slaked lime would be of more value than the combined phenols. (Protocol 9 and 10, Appendix.)

Freshly prepared calcium hydroxide has a coefficient of approximately 20 based on the fresh lime used. (Protocol 11.) It is highly efficient theoretically, but as a practical disinfectant has only a limited application, because of its insolubility, instability and because of the residue wherever applied.

The effect of lime and magnesia as they often occur in the diluting water for coal tar disinfectants and dip is of considerable importance in that the emulsifying agent or solvent is in most cases soap. One part of lime combines with 12 parts of soap forming an insoluble grease no longer capable of emulsifying or dissolving a saponaceous disinfectant. Without this constituent the active agent is no longer in a finely divided state capable of acting uniformly but separates in a layer of oil which often causes considerable damage by reason of its corrosive character in this condition. Such a water should be corrected by the use of washing soda or caustic soda. When properly treated with one or both of these ingredients the water can

be safely used as a diluent for any of the coal tar dips or disinfectants.

The comparative values of an emulsifiable and a soluble disinfectant is a question that seems entirely profitless. It has nevertheless been claimed (Chick & Martin, *Journal of Hygiene*, 1908, Vol. 8, p. 654) that the former is the more efficient since the finely divided particles of the oil were observed to have a Brownian movement and, in effect, to bombard the bacteria.

Laboratory experiments fail to verify this hypothesis. These were carried out by using cresylic acid and soap, the ratio between the two being varied to obtain in the one case a complete solution, in the other with less soap a hazy emulsion. Careful tests of these two preparations failed to show any material difference. (See Protocol 12, Appendix.)

Another point in connection with coal tar disinfectants is the effect of exposure to light.

That this effect is to lower the efficiency is the statement made by Prof. Charles E. Higgins of Ottawa (personal communication) who found a marked decrease in germicidal activity in several samples so exposed for three weeks.

To verify this statement a sample was divided, one part remaining in an amber bottle, the other in a small clear glass bottle exposed to the light, partly direct sunlight.

After one month this was found to have lost about 8 per cent. of its activity as compared with the other part. This is not a negligible loss. It is one that should be guarded against by avoiding undue exposure of disinfectants, especially the sample for assay representing a bulk lot. It may well be that this is, in part, the explanation of disagreements in reports from different tests of the same preparation.

The actual loss, however, is insignificant and is offset by the fact that recommended dilutions are usually much lower than the efficiency of the preparation would permit. (See Protocol 13, Appendix.)

One occasionally wonders what combination of circumstances is responsible for the appearance and retention on the market of certain disinfectants for which high values are claimed and which uniformly fail to show these high values by any of the accepted tests. In the case of one product of this character it is claimed to be ten times as efficient as phenol while careful tests show a value only slightly in excess of that of phenol. The active constituent appears to be B-naphtol dissolved in glycerine. (See Protocol 14, Appendix.)

Another instance of a product less valuable in general than would be justified by the claims is one which has the recommendation of the hygienic laboratory. It is even called the Hygienic Laboratory Pine Oil Disinfectant. It is prepared from a specially distilled pine oil and the impression is given that any oil so prepared is equally valuable. It is described as if it were a new product, while as a matter of fact similar preparations have been on the market for years. Tests are shown (Public Health Report, October 8, 1915) on which a coefficient of 5 is claimed

and a dilution of 1 in 500 recommended for general use while this same dilution acting on a filtered suspension of B. *typhosus* failed to kill in 15 minutes' exposure. (See Protocol 15, Appendix.)

As an evidence of the possible harm done by such a publication a sample of disinfectant prepared in North Carolina from an oil supposed to be identical with the specifications had a coefficient of 0.5. (See Protocol 16, Appendix.) This producer was justified in expecting a coefficient ten times as high as his product actually possessed and on the basis of the statement below would be justified in marketing his product and quoting as follows:

(PUBLIC HEALTH REPORTS, OCT. 8, 1915.)

"The new preparation is derived from pine oil, a by-product in the manufacture of turpentine. It is easily prepared by mixing certain portions of the oil with rosin and sodium hydroxide solution, the finished product being a reddish-brown liquid, rather thick and oily in appearance, but free from turbidity. With water it makes a perfectly white emulsion, much resembling milk. It has a pleasing odor, no objectionable taste, and attacks neither fabrics nor metals. It possesses over four times the disinfectant properties of carbolic acid and is altogether non-toxic, so that it may safely be used as a throat spray or mouth wash in solutions of the ordinary strength. The cost of the preparation is remarkably low, as it can be manufactured for less than fifty cents a gallon, solely from products which are produced in this country.

"It is believed that this new compound, which is to be known as "Hygienic Laboratory Pine-Oil Disinfectant," will become one of the most useful preparations of that character. Fortunately the high cost of the oil has prevented the flooding of the market with this low grade disinfectant."

A brighter picture is presented by Dakin who brought into use a valuable form of hypochlorite. While the solution Eusol and the powder Eupad suggested by him have no extraordinary values, the toluene derivative, Chloramine T, has properties which commend it to our attention. No extended examination has been made to determine its general applicability, but germicidal assays by the accepted methods show its value to be 50. (See Protocol 17, Appendix.) The presence of organic matter materially lowers this value, but when used as suggested by Dakin, it is probably a safe disinfectant.

On many occasions there is need for a preservative agent to prevent the bacterial decomposition of solutions of organic substances or drug and glandular extracts. While ethyl alcohol is more or less ideal for this purpose, in some cases it is not applicable either because of its action on an active ingredient or because of its effect on the patient.

Certain of the phenols derived from coal tar are made use of, especially in antitoxin. Where those commonly used are not applicable because of the irritation to tissues, other phenols from

high coefficient oil can be substituted to advantage. Their low toxicity, freedom from irritation and high antiseptic values all tending to make them very valuable. (Protocol 18.)

Another substance applicable for this purpose is Chloretone—trichlor-tertiary-butyl alcohol. A saturated solution—about 0.8 per cent.—kills B. *typhosus* after two minutes. It is less effective against spores and moulds but is almost universally applicable against bacterial decomposition. (Protocol 19.)

When the bacteriologists discovered that certain dyes have a specific staining action on bacteria it was the first step toward applying these dyes as germicidal or at least antiseptic agents. There seems to be no relation between color and germicidal activity but, regardless of color, the acid dyes appear to be inert and the basic, active.

No exhaustive experiments have been made to determine the value of these substances as disinfectants but experiments carried out by Churchman (*Journal Experimental Medicine*, Vol. 16, 1912, p. 221), Russell (Same Journal, Vol. 20, 1914, p. 545) Hill and Tabor (*Journal Infectious Diseases*, Vol. 15, 1914, p. 566) show a high degree of antiseptic value.

The low toxicity and freedom from irritation of some of the promising dye products lead to some experiments which showed that in vitro methyl violet has a phenol coefficient of 200 against Bact. *diphtheriæ*. Other dyes, gentian violet, malachite green, methylene blue, showed toward Bact. *diphtheria* and B. *typhosus* no remarkable value. (Protocol 20.) When the price of dyes resumes a reasonable level it seems very probable that some selected ones may be found well adapted to internal antisepsis and especially in attempting to control diphtheria and typhoid carriers.

In some cases it seems probable that, while certain of these dyes are strongly antiseptic in vitro, they are not exceptional germicides and, therefore, in the living animal are almost valueless. Unless the micro-organism is killed favorable conditions for growth are very soon resumed.

This is to a degree applicable to the chemical substance known as Chinosol which appears on the market also under the name Pix Cresol (*Journal American Medical Association*, 1911). While it actually has exceptionally high antiseptic value—many times more powerful than phenol—its germicidal value is almost negligible, and it finds little practical use.

To summarize the conclusions that may be drawn from the foregoing we find:

First, formaldehyde, when used under conditions in which the gas acts in an aqueous solution, is an efficient disinfectant, probably the only generally applicable fumigant.

Second, mercuric iodide combined with potassium iodide is an exceptionally valuable germicide for the skin and for disinfecting surgical instruments and vessels. Its germicidal value is 5,000 times as high as that of phenol.

Third, lime water freshly slaked from good stone lime has high germicidal value, but its objectionable features both in itself and in its action on

other disinfectants makes its use more or less detrimental.

Fourth, soap can be considered valuable only as an aid to disinfection or as a vehicle for an active agent. Most disinfectants with the exception of mercuric-potassium iodide add little to the efficiency of soap.

Fifth, alcohol as commonly used, *i.e.*, on the skin previously washed with soap and water is highly efficient, in percentages of 30 or over.

Sixth, a coal tar disinfectant carefully standardized by one of the accepted methods is valuable in proportion to its phenol coefficient and is less affected than some others by the factors which tend to inhibit the value of a germicide.

Seventh, pine oil disinfectants and other special products require as careful standardization as the coal tar disinfectants. For safe use the dilution should not exceed a number representing the result of the phenol coefficient, times the dilution of pure carbolic acid which would be used with confidence under similar conditions.

Eight, certain of the aniline dyes give promise of practical value in wounds and as internal antiseptics because of having a selective action toward certain microörganisms and because of their low toxicity.

The old dependable germicides, mercuric chloride, phenol, iodine, hydrogen peroxide, will never be displaced, but undoubtedly their field of usefulness will become more and more restricted. The trend of future research in the field of disinfection leads toward the development of specific germicidal agents.

Appendix.

Protocol 1. From original notes.

Silver compound Tested by Hygienic Laboratory Method.

	Medium Adjusted to 1.5 acidity.	
Dilutions.	*2½ min.*	*5 min.*
1–25	−	−
50	−	−
75	−	−
100	−	−
150	−	−
200	−	−
250	−	−
300	−	−
400	−	−
500	−	−
600	+	−

	Medium not adjusted—acidity 0.8.	
	2½ min.	*15 min.*
1–25	−	−
50	+	+
75	+	+
100	+	+
150	+	+
200	+	+
250	+	+
300	+	+
400	+	+
500	+	+

Protocol 2. From original notes.

Formaldehyde gas generated in a space of 800,000 cc. capacity.

54 cc. failed to kill bedbugs and roaches.

16 cc. failed to kill flies and moths.

These quantities exceed the amount necessary for efficient fumigation which is 13 cc. for this space. The experiments with bedbugs and roaches were continued with increasing amounts to determine if any quantity is toxic in vapor form.

The solution is said to be more or less toxic to flies when ingested, but is

by no means a specific poison when so used.

Protocol 3. From Bul. No. 27. Hygienic Laboratory.

Experiment 4.

Formalin used	600 cc.
Permanganate used	300 gm.
Capacity of room	2,000 cu. ft
Yield of formaldehyde	33%
Temperature	77°F.
Relative humidity	72%

Results	5	10	15	20	30	45	60
B. pyocyaneous	+	+	−	−	−	−	−
B. coli communis	+	−	−	−	−	−	−
B. dysenteriæ	+	+	−	−	−	−	−
B. subtilis	+	+	+	+	+	+	−

Experiment 6.

Formalin used	600 cc.
Permanganate used	300 gm.
Capacity of room	2,000 cu. ft
Yield of formaldehyde	37.7%
Temperature	71°F.
Relative humidity	45%

Results	5	10	20	45	60	90	120
B. pyocyaneous	+	+	+	+	−	−	−
B. coli communis	+	+	+	−	−	−	−
B. dysenteriæ	+	+	+	+	−	−	−
B. subtilis	+	+	+	+	+	+	+

Difference due to percentage of humidity.

Protocol 4. From original notes.

Germicidal tests of samples of Castile soap. Sample 1–6 inclusive were practically neutral. Sample No. 8 was distinctly more alkaline than No. 7 and both contained decidedly more alkali than the others.

Sample phenol.	*Coefficient.*
1	.05
2	less than .05
3	less than .05
4	.07
5	.05
6	.05
7	.18
8	.26

Adding 1 part KOH to 30 parts of No. 4, the resulting mixture had a coefficient of .3.

Protocol 5. From "Soaps from different glycerides," Hamilton. *J. Ind. & Eng. Chem.*, Aug. 1911.

Test organism—B. typhosus.

\+ indicates growth in subculture.
− indicates no growth in subculture.

Glyceride.	*Oil used.*	*Dilution.*	*Result in 5 min.*
Trilaurin	Cocoanut	1–40	−
		1–50	+
Tribrassin	Rape seed	1–20	+
Trivalerin	Whale	1–20	+
Trilinolein	Linseed	1–20	+
Triricinolein	Castor	1–20	−
		1–30	+
Tripalmitin	Palm	1–20	+
Triolein	Olive	1–20	−
		1–30	+
	Resin	1–20	+

Protocol 6. From "Germicidal Value of Ethyl Alcohol," Harrington & Walker, *Boston Medical and Surgical Journal*, Vol. 148, 1903, p. 548.

B. typhosus—Dried. Alcohol	5	10	15	30	45	60 min.
15%	+	+	+	+	+	+
20	+	+	+	+	+	+
25	+	−	−	−	−	−
30	−	−	−	−	−	−
80	−	−	−	−	−	−
85	+	+	+	−	−	−
90	+	+	+	+	+	+
B. typhosus—Moist.						
15	+	+	+	+	+	+
20	+	+	+	+	+	+
25	+	−	−	−	−	−
30	−	−	−	−	−	−
90	−	−	−	−	−	−
99	−	−	−	−	−	−

From original notes.

	1	2	3	4	5	min.
25	+	+	+	+	+	
30	+	+	+	+	+	
40	−	−	−	−	−	
85	−	−	−	−	−	
90	+	−	−	−	−	

Protocol 7. Phenol coefficients claimed for a commercial product.

Toward	B. pestis	40
	B. diphtheria	30
	B. entericus	20
	B. coli	17
	Cholera	42

From original notes.

Coefficients of two disinfectants based on two different organisms.

	B. typhosus.	*B. pyocyaneous.*
Disinfectant 1	4	2
" 2	16	4

Protocol 8. From original notes.

Phenols separated from coal tar oil and redistilled to further separate at each 10° C. or at such points as to obtain sufficient for germicidal test.

Emulsive disinfectants containing 78 per cent. of oil were made from each.

Boiling points.	*Coefficients.*
200°–210° C.	5.5
210°–220° C.	8.7
220°–230° C.	14.5
230°–240° C.	18.1
240°–280° C.	24.

The entire phenols made into a similar product had a coefficient of 17.

Ordinary cresylic acid with boiling points of 185°–205° has a coefficient of 4–5.

Protocol 9. From original notes.

Phenol in aqueous solution.

	2½ min.	*15 min.*
1- 90	−	−
1-100	+	−
1-110	+	−
1-120	+	+

Phenol molecularly combined with caustic soda.

1-20	−	−
1-40	−	−
50	+	−
60	+	+
80	+	+

Phenol molecularly combined with lime.

1-20	−	−
40	+	+
60	+	+
80	+	+

Protocol 10. From original notes.

Cresylic acid and soap.

[Cresol Compound U. S. P.] containing 50 per cent. cresols.

Dilutions.	*2½ min.*	*15 min.*
1-180	−	−
200	+	−
220	+	−
240	+	+

Cresylic acid combined molecularly with lime.

Dilutions based on cresol content.

1-175	−	−
200	−	−
250	+	−
300	+	+
350	+	+

Cresylic acid combined molecularly with caustic soda.

1-160	−	−
200	+	−
240	+	+
280	+	+

Coefficients based on the cresol content.

1st	approximately	4
2nd	"	2.4
3rd	"	2

Protocol 11. From original notes.

Germicidal assay of freshly slaked lime.

Dilutions based on the lime (CaO) used.

Dilutions.	*15*	*30*	*45*	*60*	*min.*
1–1,000	–	–	–	–	
1,500	–	–	–	–	
2,000	–	–	–	–	
2,500	+	–	–	–	
3,000	+	+	+	+	
4,000	+	+	+	+	
Phenol dilutions.					
1–120	–	–	–	–	
140	+	–	–	–	
160	+	+	+	–	
180	+	+	+	+	

Coefficient about 20.

Protocol 12. From original notes.

Disinfectant No. A, about 80 per cent. Cresylic Acid, [Cresylic Acid 8 cc., Soft Soap 2 gm.]

Disinfectant No. B, about 50 per cent. Cresylic Acid, [Cresylic Acid 5 cc., Soft Soap. 5 gm.]

HYGIENIC LABORATORY METHOD.

Dilutions.	*Results.*			
No. 1 and No. 2.	2½ *min.*		15 *min.*	
	A	B	A	B
300	–	–		
400	–	+		
450	–	+		
500	–	+		
550	–			–
600	–			–
650	+		–	+
700	+		–	+
750			–	+
800			–	
850			+	

Phenol.		
90	–	
100	+	
110		–
120		+

Coefficient A 6.9.
B 4.6.

When calculated to the cresylic acid on the basis the amount contained in each

Coefficient A–8.6.
B–9.2.

Protocol 13. From original notes.

GERMICIDAL ASSAY—HYGIENIC LABORATORY METHOD.

Sample of disinfectant that has been exposed to the light August 28–September 19–28. (1) compared with the original sample (2).

		2½ *min.*				2½ *min.*	
	1–1,200	–			1–1,200	–	
	1,400	+			1,400	–	
(1)	1,600	+	–	(2)	1,600	–	–
	1,800	+	–		1,800	+	–
	2,000		–		2,000		–
	2,200		+		2,200		+
	1–1,200	–			1–1,200	–	
	1,400	–			1,400	–	
	1,600	+	–		1,600	–	–
	1,800	+	+		1,800	+	–
	2,000		+		2,000		+
	2,200		+		2,200		+
	1–1,400	–	–		1–1,400	–	–
	1,600	+	–		1,600	–	–
	1,800	+	+		1,800	+	–
	2,000	+	+		2,000	+	+
	2,200	+	+		2,200	+	+

Phenol.		
1–100	–	–
110	+	–
120	+	–
130	+	+
140	+	+

Coefficient (1) 13.6.
" (2) 15.

Protocol 14. From label on package of disinfectant. "Leaves no odor. A powerful antiseptic and germicide of the Naphthalene series."

Inert Ingredients.
Water 23 per cent.
Glycerine 40 per cent.

"Seven times less poisonous and ten times more efficient than carbolic acid."

From results of assay

Dilutions.	2½	5 *min.*
1–100	–	–
175	+	–
200	+	–
250	+	+
300	+	+
400	+	+
600	+	+
800	+	+
1,000	+	+
Phenol.		
1–110	–	–
120	+	–

Coefficient 1.6.

Protocol 15. From Public Health Reports, Oct. 8, 1915, p. 3008.

B. typhosus, 20° C.

Phenol.	2½	15 *min.*
80	–	–
90	+	–
100	+	–
110	+	+
120	+	+
Pine Oil Disinfectant.		
375	–	–
400	–	–
450	+	–
500	+	+
550	+	+

Coefficient 4.75.

Note growth from 500 dilution in 15 min. subculture.

Protocol 16. From original notes. Pine Oil Disinfectant. From John A. MacKeathan, Fayetteville, N. C.

Hygienic Laboratory Method.

Dilutions.	2½	15 *min.*
1st test.		
1–150	+	–
200	+	–
300	+	+
400	+	+
500	+	+
600	+	+
2d test.		
Dilutions.	2½ *min.*	15 *min.*
1– 50	–	
75	+	
100	+	+
125	+	+
150	+	+
200	+	+
3d test.		
1– 30	–	
40	–	
50	–	–
75	+	–
100	+	+
125		+
Phenol.		
1–100	–	
110	+	–
120		–
130		+

Coefficient .55.

Protocol 17. From original notes.

Chloramine Assay—Hygienic Laboratory Method.

	2½ *min.*	15 *min.*
1,800	–	
2,000	–	
2,200	+	
2,400	+	–
2,600	+	–
5,000		–
6,000		–
8,000		–
10,000		–
12,000		+

Coefficient about 50. This test shows how important a factor time is in testing the value of Chloramine.

Protocol 18. From original notes.

Germicidal test of a mixture of water soluble phenols applicable where freedom from toxicity and irritation combined with high efficiency are essential factors.

Dilution.	*Time and Results.*	
	2½ min.	*15 min.*
1,800	−	
2,000	−	−
2,200	+	−
2,400	+	−
2,600	+	+
Phenol.		
90	−	
100	−	
110	+	−
120		−
130		+

Coefficient 20.

Protocol 19. From original notes.

Germicidal value of a saturated solution of Chloretone in distilled water (about 0.8 per cent.). The solution was inoculated with a culture of B. *typhosus* and sub-cultures taken at 1 minute intervals as follows:—

1	2	3	4	5	6	7	8	9	10
+	−	−	−	−	−	−	−	−	−

Protocol 20. From original notes.

Germicidal test of Methyl Violet against B. *diphtheria*. Sub-cultures made at 1 minute only.

Dilutions.	*1 min. Sub-cultures.*
1,000	−
2,000	−
5,000	−
10,000	−
15,000	−
17,000	−
18,000	+
20,000	+

Test of Gentian Violet under the same conditions.

Dilutions.	*1 min. Sub-cultures.*
1,000	+
5,000	+
10,000	+
20,000	+
50,000	+

Test of Malachite Green under same conditions.

Dilutions.	*1 min. Sub-cultures.*
500	−
1,000	−
1,200	−
1,400	+
1,600	+
2,000	+